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33. *Nica Cadma*. I took this once in April, in a pasture behind Bluefields, and two or perhaps three times, on the Hampstead Road in June and July. When in good condition the purple flush upon the red of the posterior wings is very rich and beautiful.

34. *Eubagis Zetes*. In the month of June I first met with this little butterfly about midway between Bluefields and Savanna le mer; two or three specimens then occurred, not exactly associated, and yet not far from each other. They were playing about some shrubs by the road-side and were easily caught. A few others occurred in July, after which I saw it no more. I think on all occasions that I met with the species, which were but few, I found several at no great distance from each other. It seems a lowland species, and limited in the season of its activity to the middle of summer.

[To be continued.]

XX.—*An Account of the Germination of Isoëtes lacustris.*
By KARL MÜLLER*.

[With two Plates.]

[Continued from p. 93.]

5. *From the epoch when the Germ-plant breaks through to the formation of the second Leaflet.*

IN the germinal body (*Keimkörper*) may be distinguished: 1, the reservoir for nutrient matter mentioned in foregoing paragraphs. To facilitate the descriptions I will call this the *alimentary organ* (*Nahrungsspeicher*); 2, *the matrix of the root*; 3, *the vagina*; 4, *the scale of the first leaf*; 5, *the cell of the second leaf*, or to describe it in more general terms, the point of gemmation for all the following leaves; 6, *the first leaf*. I shall have to consider these in a somewhat different order, in accordance with the development of the plant.

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1. *The Alimentary Organ*.—This part of the germinal body

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first becomes distinctly visible in the stages just preceding the breaking-through of the ovule (Pl. II. fig. 15 *c*). It here lies close to the matrix of the root (fig. 15 *f*), which at this period it wholly resembles in shape. Each of these constitutes one-half of the germinal body, the matrix of the root that one on which the vagina of the leaf-bud occurs, the alimentary organ the opposite. Upon the two rests the first leaf, so that the vagina is pushed a little to one side.

The alimentary organ is composed of very small, parenchymatous cells, which are so densely filled with granular contents that the organ is clearly defined, in contrast with the matrix of the root, as a darker-coloured body. The manner in which this kind of cell-contents is restricted to the cells of one-half of the germinal body is exceedingly striking. This matter takes a deep blue colour with iodine, and thus clearly displays its amylaceous nature. By means of this reagent also we find that the amyllum has not been deposited all at once in this region. Even in much earlier stages, for instance in fig. 11 *a*, the formation of it has commenced, and it continues gradually from that time till the alimentary organ is perceptible as an independent part of the germinal body.

Amyllum-cells are indeed found in very early stages in other parts of the embryo, for example in fig. 11 at *d* also; but at this point they become rapidly absorbed and applied to the further development of the embryo. As for the rest, the amyllum originates from one and the same matrix in all parts of the plant, namely from the cytoblast. This is but a new confirmation of the observations which I had an opportunity of making formerly in *Chara crinita**, and which have been confirmed by English botanists, especially by Quekett†. It here appears that single cytoblasts become decomposed into many starch granules (Pl. II. fig. 17 *f*, Pl. III. figs. 18 *d*, 20 *f*, 21 *c*), since in those cells where the starch granules occur, the cytoblasts have wholly disappeared, and yet no other matter is present. This agrees also with the formation of chlorophylle; for in those cells where this occurs, nothing but cytoblasts are to be found at an earlier period. These suddenly vanish and a quantity of granules appear in their place, which at first acquire a blue colour with iodine, and consequently have been transformed into starch; subsequently they become perfectly green.

By degrees, accompanying strictly the gradual development of the germinal body, the starch of the alimentary body undergoes

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solution. It does not wholly disappear until the time when the second leaflet emerges from the vagina and acquires a green colour. Then the cells of the alimentary body which have step by step become looser and more transparent are found quite empty (figs. 22 c, 23 c), and their membranes of a brown colour.

I have never observed them to become again filled with any kind of matter. It appears to me probable, that we have here, on a small scale, what afterwards happens on a large one in the rhizome of *Isoëtes*, where the outermost layers, so soon as they have fulfilled their functions in the service of the leaves, when these decay are also destroyed, since they are then to be met with only as a brown spongy tissue forming the brown lamellæ on each side of the rhizome.

2. The *Matrix of the Root*.—Although it is impossible to perceive where the first root will be developed, in the stage where the matrix and the alimentary body have exactly the same form (fig. 15 c, f), there is no doubt about the matter in the stages of development which soon succeed, since the root now unfolds very rapidly.

In this we find conditions exactly opposed to what occur in the alimentary body. The matrix of the root is composed of the same small parenchymatous cells. As in that, these originally possess distinct cytoblasts. But unlike what we found there, these do not become transformed into starch, but are dissolved at once into an almost transparent cytoblastema. This is the first stage of the development of the root; by this the matrix obtains nutrient matter, by means of which its further unfolding is rendered possible. The formation of new structures takes place here, as may usually be seen very distinctly in all rootlets, at the apex. And it must be noticed that the cytoblasts are first dissolved at the very apex of the matrix, this process gradually extending to the remaining portion (fig. 17 e, g). The primordial cells of the apex of the rootlet, which are of extremely delicate texture, appear empty at this stage.

An important alteration soon succeeds. All the cells of the matrix are empty, while this has become elongated gradually and in such a manner that the apex appears rather thin and the back part thicker and club-shaped (fig. 18 e). The most external layer of the cells of the matrix may also be easily distinguished from the internal. The former have become firmer and inclose the latter as an epidermis. It is striking here that the cells of its extreme point contain cytoblasts within them (fig. 18 g).

The most essential change however that occurs in this stage is the first appearance of the vessels (fig. 18 f). These emerge as two short cords from the interior of the alimentary body, and I have never been able to discover the point whence they originate.

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In *Selaginella* this may be seen very distinctly, as it manifests itself as a darker point in the interior of the germinal body. Perhaps a similar condition may exist here. The vessels themselves are darker where they emerge from the interior of the alimentary body; they run gradually out into the more anterior cells of the matrix, becoming continually more transparent.

In all the stages up to this point the root still remains inside the coat of the nucleus. Now however it breaks through this. Its escape takes place exactly opposite to the point where the first leaflet emerged (fig. 20 *g*). If the plant is extricated from the coat of the nucleus in this stage, it presents the appearance shown in fig. 20 *c*. We have here no longer any doubt of the existence of a root; it has become considerably elongated, but internally it has not altered essentially since the stage of fig. 18, where it still remained within the ovule. The external layer of cells still appears in the likeness of an epidermis, the internal cells have not altered in size or form, the point of vegetation at the apex remains still composed of the small-celled tissue, and the vessels alone have become elongated.

This condition however does not persist long; for as we pass next, in fig. 21 *a*, to a stage where the second leaflet begins to emerge from the vagina (fig. 21 *d*), in the first place the epidermoid, external layer of cells has disappeared, since the membranes of the internal cells having acquired greater consistence, no distinction between the outer and inner cells now exists. Moreover the cells are empty and transparent, differing from what was the case in the former stages, where they were always densely filled with a very delicate, clear cytoblastema; only the cells of the point of vegetation, situated at the apex of the root, are now provided with cytoblastema. Since these cells are here still small and extremely delicate, the cells of the outermost layer, which are of firmer texture, again appear like an epidermis (fig. 21 *e*), which has now altogether ceased to be the case with those cells lying further back. These latter are now much elongated in proportion to what they were in the former stages, and the result of this is an elongation of the whole root generally. This explains why they are quite empty, since their contents have been applied in all probability to the enlargement of the membranes.

The vessels have equally undergone a change. These are no longer seen to run as two distinct cords through the interior layers of the cells of the root; they are rather blended into a single mass which now runs almost to the point of vegetation of the root. But notwithstanding this combination, each vascular bundle runs forward in an independent condition. If one of these is extracted from the root, it appears, in the shape of the figure 21 *b*, as a longish utricle of an extremely delicate, hyaline

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membrane. At the extremity by which it penetrates through the cellular tissue of the leaf, it appeared to me to end in a point, while the other portion which extends through the root ended abruptly. The most interesting matter to me in the vessels was the formation of the spiral fibre. The rudiments of this consist of a number of hyaline granules, which originally have a quincuncial arrangement (fig. 21 *f*), but subsequently become blended into rows in the form afterwards presented by the spiral band. I never saw more than *three* globules in one plane, which was wholly in focus at once beneath the microscope, and comprised about one-half of the utricle of the vessel, and these lay one upon another in various directions. Consequently six of these globules must belong to each turn of the spiral. In my case, as in that of many others, all observations have failed to demonstrate the origin of the vessel. So far as I have seen, the vessel could never be reduced to a cell, since cross septa could never be discovered in it. It appeared to me almost as if the utricle of the vessel, having its origin in the alimentary body or between it and the matrix of the root, went forth at once as an independent utricle into the leaf and root, for it could not be difficult at this time for it to penetrate through the tissue of the leaf and root, since this was extremely delicate and mucilaginous at the first origin. However nothing definite can be said upon this point. But on the other hand, the origin of the spiral fibre is easier to trace here. It agrees in every respect with what I observed in *Selaginella*, and I have nothing further to add upon the subject. Subsequently, when the second leaflet has begun to rise out of the vagina, the vascular bundle divides into two (fig. 22), each of which is composed of two cords.

3. The *First Leaf*.—The same events which occur at the lower part of the germinal body, in the root, take place above in the first leaf. The two organs are parallel with each other in many respects. The formation of the vascular bundle is exactly the same here. The cells, too, are at first small, delicate and parenchymatous, wholly as in the root. Thus they are when the leaf breaks through the coat of the nucleus. The next elongation of the leaf occurs by the expansion of these cells. Thereby they become longer but of a paler green (fig. 17 *a*), having previously had a very beautiful deep green colour (fig. 16 *b*). Not until much later does the elongation of the leaf appear to result from the formation of new cells, which in all probability occurs by division, since we never see a point of vegetation at the apex of the leaf like that of the root. Neither have I observed it at the base.

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There is little to be said concerning the leaf itself. When it has emerged from the ovule, it always has a curved, often a hooked direction.

The most interesting point about this organ is, that, after the coat of the nucleus is burst, the germ-plant no longer remains at the bottom of the ovule, and the leaf has not merely part of its apex but almost its whole length situated outside the opening (figs. 16, 17, where the drawing exhibits the condition more accurately). At a subsequent period only does the germ-plant reach the bottom of the ovule by means of the root, running downward and piercing through the coat of the nucleus below (fig. 20 *g*). This rising of the germ-plant in the nucleus certainly depends upon its being specifically lighter, the germinal body evidently not being heavy enough to retain the plant at the bottom of the nucleus.

It has already been mentioned above (§ 4.), that the future furrow of the leaf exists in a rudimentary condition very early, as a fold, in the embryo.

4. The *Leaf-scale*.—Of this also it has been previously seen that its rudiments are formed very early, since the mother-cell of it appears in that stage when the separation of the germinal body into alimentary organ, root, vagina, &c. scarcely exists (fig. 11 *c*). As such it is seen to remain for a long time, without essential alteration of its form (figs. 11 *c*, 12 *f*, 13 *d*, 15 *d*). In the mean time however a process of cell-formation has taken place similar to that previously occurring in the mother-cell of the embryo. New cells have been formed. By this means the shape of the mother-cell has been somewhat changed; it has become a body densely filled with cellular tissue. This stage of the scale is found in that germ-plant which is represented in Pl. II. fig. 15. The scale which previously had the appearance of a globule has now become a smooth disc which stands close against the cell of the second leaf lying below it (figs. 16 *c*, 17 *c*).

During the continuous growth of the germ-plant it attains its highest stage of development, when the vessel of the first root begins to show itself (Pl. III. fig. 18 *h*.) It is then a flat, broad, more or less reniform disc which is somewhat folded together, so that the succeeding leaflet is almost entirely surrounded by it, whence one might be led to imagine that it only existed on account of its connexion with that succeeding leaflet.

It is composed of very delicate parenchymatous cells which contain nothing in the young condition of the plant, when the cytoblasts of the cellular tissue have disappeared, though sometimes these are to be found persisting for a longer time. The scale is often toothed on the border with extraordinary regularity. In such cases one cell projects from the border as a sharp tooth while the next remains unextended, and then the third cell again projects as the second tooth. I have however also found the scale very irregularly toothed. This is indeed a

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disc, but not rounded off like the former, rather apiculate above, and furnished with horn-like projecting cells of considerable size.

The second scale, that of the second leaflet, is also a disc, and the one which I found as such deviated again from the forms of both the preceding, being of a more or less oval shape and furnished with some minute projecting cells at the apex.

By the sight of these scales I was reminded instantly of that accessory organ which I found in the *Selaginella*, and of which I have given a complete history of development. I at once concluded these to be of the same morphological import. In a treatise by Mettenius on *Azolla** which appeared just at that time, he compared the scale of *Isoëtes* with a *ligule*, while he referred the organ of the *Selaginella* to an axillary *stipule*. I will not venture to decide either for the one or the other, but I think, as I have said, that they are of similar origin and also of similar import.

To complete the characterization of the scale I will add the description of Mettenius (*op. cit.*), since this observer has examined it on the mature plant, which I have not:—"In *Isoëtes* this scale occurs on the fertile and barren leaves. In young leaves it is seated on the surface, in old ones apparently in an excavation, since its lower part is ensheathed by the substance of the leaf, and only the upper scale-like portion lies free upon the inner face of the leaf. The point of attachment of the scale is broader than the proximate ensheathed portion, and is formed of a cellular tissue distinctly defined from the parenchyma of the leaf. The nerve of the leaf runs up behind the point of attachment, and the cellular tissue surrounding this contains numerous spiral-fibrous cells, which extend down to the point of attachment of the sporangium."

5. The *Vagina*.—We found the first rudiment of this in the stage of fig. 11 *b*; there were then only isolated spherical cells, elevated above the surface indicating the vagina; subsequently they are raised up with the matrix of the root in such a manner that they are now connected in an uninterrupted line with the furrow of the first leaflet (figs. 12 *d*, 13 *c*, 15 *g*). At this time these single cells surround, as a cup, the second leaflet projecting out beyond them (figs. 17 *h*, 18 *i*, 20 *h*). They then become multiplied by division, and thus very delicate and minute, so that this tissue is now distinguished in a marked manner from that surrounding, by its greater delicacy (fig. 21 *g*). Thereby it may be observed that a peculiar delicate membrane, composed of a simple layer of cells, is in course of formation.

* Linnæa, 1847, p. 270.

disc, but not rounded off like the former, rather apiculate above, and furnished with horn-like projecting cells of considerable size.

The second scale, that of the second leaflet, is also a disc, and the one which I found as such deviated again from the forms of both the preceding, being of a more or less oval shape and furnished with some minute projecting cells at the apex.

By the sight of these scales I was reminded instantly of that accessory organ which I found in the *Selaginella*, and of which I have given a complete history of development. I at once concluded these to be of the same morphological import. In a treatise by Mettenius on *Azolla** which appeared just at that time, he compared the scale of *Isoëtes* with a *ligule*, while he referred the organ of the *Selaginella* to an axillary *stipule*. I will not venture to decide either for the one or the other, but I think, as I have said, that they are of similar origin and also of similar import.

To complete the characterization of the scale I will add the description of Mettenius (*op. cit.*), since this observer has examined it on the mature plant, which I have not:—"In *Isoëtes* this scale occurs on the fertile and barren leaves. In young leaves it is seated on the surface, in old ones apparently in an excavation, since its lower part is ensheathed by the substance of the leaf, and only the upper scale-like portion lies free upon the inner face of the leaf. The point of attachment of the scale is broader than the proximate ensheathed portion, and is formed of a cellular tissue distinctly defined from the parenchyma of the leaf. The nerve of the leaf runs up behind the point of attachment, and the cellular tissue surrounding this contains numerous spiral-fibrous cells, which extend down to the point of attachment of the sporangium."

5. The *Vagina*.—We found the first rudiment of this in the stage of fig. 11 *b*; there were then only isolated spherical cells, elevated above the surface indicating the vagina; subsequently they are raised up with the matrix of the root in such a manner that they are now connected in an uninterrupted line with the furrow of the first leaflet (figs. 12 *d*, 13 *c*, 15 *g*). At this time these single cells surround, as a cup, the second leaflet projecting out beyond them (figs. 17 *h*, 18 *i*, 20 *h*). They then become multiplied by division, and thus very delicate and minute, so that this tissue is now distinguished in a marked manner from that surrounding, by its greater delicacy (fig. 21 *g*). Thereby it may be observed that a peculiar delicate membrane, composed of a simple layer of cells, is in course of formation.

* Linnæa, 1847, p. 270.

This is very apparent when the second leaflet has become considerably elongated beyond the margin of the vagina (figs. 22 *d*, 23 *d*), and it is most closely connected with the furrow of the first leaf. Looked at from this point, the vagina appears abruptly cut off.

By the time the third leaflet appears, the vagina has vanished and the bases of the two first leaves surround the third as a sheath, as is the case in the full-grown plant (fig. 24 *d*).

6. The *Second Leaf*.—The rudiment of this also is formed at a very early epoch, and in fact at the time when the first leaf is still a mother-cell. Consequently two mother-cells, for two different organs, occur close together in the vagina, which itself is in the earliest stage of formation (figs. 12 *c*, 13 *b*, 15 *e*, 16 *d*).

While the mother-cell of the scale grows into a disc-shaped body, the mother-cell of the second leaf expands more and more in a globular form. It is soon observed that, already in this form, cellular tissue has been produced in its interior (fig. 18). This epoch is contemporaneous with the perfect formation of the scale of the first leaf. From this cause the vagina has become drawn so closely round the second leaflet (figs. 18 *i*, 20 *h*), that the latter is ensheathed in it, as an obtuse conical body.

Very soon, however, the first leaflet rises out far beyond the vagina, at the same period when this has acquired a rather delicate membrane (fig. 21 *d*, and the vagina *g*). The leaflet is by this time green, and when seen from the side, as in fig. 21 *d*, it appears somewhat compressed on two sides. No great changes go on meanwhile in the second leaflet until it emerges from the now much-elongated vagina (fig. 22 *e*). By that time the second scale has been formed at the base, inside the vagina. The most important change however has happened at the base, underneath the vagina. Here a gradually-formed protuberance displays itself, composed of very delicate cellular tissue (fig. 22 *f*). This is the rudiment of the second rootlet, or that of the second leaf. Its further development resembles that of the first rootlet. As it becomes elongated the vessels may soon be observed, of which two again display themselves here (fig. 23 *e*). The cells are at first delicate and full of cytoblastema. Subsequently, when considerable elongation has taken place, they become emptied and of longer shape, exactly as in the first rootlet, wholly as in the leaflet itself.

I have already mentioned that about this time the vascular bundle of the first rootlet has become divided into two (figs. 22, 23).

[To be continued.]

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